Molecular Biology of the Cell
Vol. 6, 777–791, July 1995

The *Drosophila* snr1 and brm Proteins Are Related to Yeast SWI/SNF Proteins and Are Components of a Large Protein Complex

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Submitted March 9, 1995; Accepted April 14, 1995
Monitoring Editor: Judith Kimble

During most of *Drosophila* development the regulation of homeotic gene transcription is controlled by two groups of regulatory genes, the trithorax group of activators and the Polycomb group of repressors. *brahma* (*brm*), a member of the trithorax group, encodes a protein related to the yeast SWI2/SNF2 protein, a subunit of a protein complex that assists sequence-specific activator proteins by alleviating the repressive effects of chromatin. To learn more about the molecular mechanisms underlying the regulation of homeotic gene transcription, we have investigated whether a similar complex exists in flies. We identified the *Drosophila snr1* gene, a potential homologue of the yeast *SNF5* gene that encodes a subunit of the yeast SWI/SNF complex. The *snr1* gene is essential and genetically interacts with *brm* and *trithorax* (*trx*), suggesting cooperation in regulating homeotic gene transcription. The spatial and temporal patterns of expression of *snr1* are similar to those of *brm*. The snr1 and brm proteins are present in a large ($>2 \times 10^6$ Da) complex, and they co-immunoprecipitate from *Drosophila* extracts. These findings provide direct evidence for conservation of the SWI/SNF complex in higher eucaryotes and suggest that the *Drosophila* brm/snr1 complex plays an important role in maintaining homeotic gene transcription during development by counteracting the repressive effects of chromatin.

INTRODUCTION

The specification and maintenance of cell fates is critical to the development of multicellular organisms. One class of genes that plays critical roles in this process, the homeotic genes of the Antennapedia complex (ANT-C) and the bithorax complex (BX-C), encode homeodomain-containing transcription factors that determine the identities of segments along the body axis in *Drosophila* (Duncan, 1987; Kaufman *et al.*, 1990) and in other animals (Kenyon, 1994; Krumlauf, 1994). The transcription of ANT-C and BX-C genes must be regulated precisely during development, as their misexpression can lead to dramatic alterations in cell fate. Relatively early in embryogenesis, the initial patterns of homeotic gene transcription are established by DNA-binding regulatory proteins encoded by segmentation genes (for review, see Harding and Levine, 1988; Ingham, 1988). Later in development, these patterns are maintained by two opposing groups of *trans*-acting regulatory genes: the Polycomb group of repressors and the trithorax group of activators. The regulation of homeotic gene expression thus consists of two major phases: establishment by segmentation genes and maintenance by Polycomb and trithorax group genes.

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Polycomb group members (including *Polycomb, Polycomblike, Posterior sex combs, extra sex combs, polyhomeotic,* and others) repress the transcription of ANT-C and BX-C genes outside their normal domains of expression (Wedeen *et al.*, 1986; McKeon and Brock, 1991; Simon *et al.*, 1992; Paro, 1993). In contrast, the members of the trithorax group (including *trithorax, ash1, ash2, brahma,* and others) maintain the transcription of homeotic genes where they are required (Kennison, 1993). Both groups of regulatory genes are thus required to maintain the determined states of cells during development. Although the mechanisms of action of Polycomb and trithorax group proteins have not been firmly established, some appear to act by influencing chromatin structure. Several of the Polycomb group proteins are thought to form large complexes (Franke *et al.*, 1992) that can affect local higher-order chromatin structure (Messmer *et al.*, 1992; Fauvarque and Dura, 1993; Rastelli *et al.*, 1993). Furthermore, the Polycomb protein contains a short segment, the chromodomain, which is conserved in the *Drosophila* HP1 protein, a component of heterochromatin (Paro and Hogness, 1991). Based on these and other observations, it has been suggested that Polycomb, together with other Polycomb group proteins, packages inactive homeotic genes into heterochromatin-like complexes early in development, thereby preventing their subsequent transcription (Paro, 1993). In addition to their silencing effect on transcription of the homeotic genes, members of the Polycomb group have also been implicated in regulating some of the earliest zygotic transcriptional events in embryogenesis (Paro and Zink, 1992; Pelegri and Lehmann, 1994).

Recent studies of *brahma* (*brm*), a member of the trithorax group, have provided additional evidence that alterations in chromatin structure are critical for the maintenance of homeotic gene transcription. *brm* mutations strongly suppress mutations in *Polycomb* and cause developmental defects similar to those arising from the failure to express homeotic genes after embryogenesis (Kennison and Tamkun, 1988; Tamkun *et al.*, 1992; Brizuela *et al.*, 1994). A possible mechanism of action for the brm protein has been suggested by its similarity to a yeast transcriptional activator SWI2/SNF2. Both brm and SWI2/SNF2 contain six blocks of sequence similar to those found in DNA-dependent ATPases and helicases. SWI2/SNF2 is a subunit of a complex that contains at least 10 subunits, including the SWI1, SWI3, SNF5, and SNF6 proteins, and has a native molecular mass of $\sim 2 \times 10^6$ Da (Cairns *et al.*, 1994; Cote *et al.*, 1994; Peterson *et al.*, 1994). This SWI/SNF complex does not appear to bind DNA directly, but assists a wide variety of DNA-binding regulatory proteins, including GAL4, SWI5, and others, to activate the transcription of their target genes (Carlson and Laurent, 1994). Both genetic and biochemical studies have suggested that the SWI/SNF complex contributes to transcriptional activation by overcoming the repressive effects of chromatin on transcription (Hirschhorn *et al.*, 1992; Winston and Carlson, 1992).

Is a *Drosophila* counterpart of the yeast SWI/SNF complex involved in the maintenance of homeotic gene regulation, perhaps by alleviating the repressive effects of Polycomb group members? Although *brm* is the closest *Drosophila* relative of *SWI2/SNF2*, their functional relationship remains unclear. The DNA-dependent ATPase domains of the brm and SNF2/SWI2 proteins are functionally interchangeable (Elfring *et al.*, 1994); it is thus likely that brm and SWI2/SNF2 play similar roles in transcriptional activation. However, the *brm* gene is unable to complement a *swi2/snf2* null mutation in yeast (Elfring *et al.*, 1994), suggesting that there may be important differences between the two proteins. Consistent with this possibility, the brm and SWI2/SNF2 proteins are not highly related outside the DNA-dependent ATPase domain; these divergent regions are thought to contribute to the functional specificity of SWI2/SNF2 family members by mediating interactions with other proteins.

To further explore the role of *brm* in homeotic gene regulation, we examined whether the brm protein is part of a *Drosophila* counterpart of the yeast SWI/SNF complex. We also searched for additional *Drosophila* relatives of yeast genes encoding components of the SWI/SNF complex. Our initial attempts to identify *Drosophila* homologues of the yeast *SNF5* and *SNF6* genes by low-stringency hybridization and by complementation of null mutants were unsuccessful (Dingwall and Scott, unpublished results). As an alternative approach, we searched for *Drosophila* genes related to *ini1*, a distant human relative of the yeast *SNF5* gene (Kalpana *et al.*, 1994). The *ini1* gene was recently identified in a yeast two-hybrid screen for proteins that directly interact with HIV integrase. The ini1 protein activates transcription of a GAL1-*lacZ* reporter when it is tethered to DNA via a GAL4 DNA binding domain, suggesting that *ini1* may also be involved in transcriptional activation (Kalpana *et al.*, 1994). In this report, we describe the identification and characterization of a *Drosophila* relative of *ini1*, that we have named *snr1*, for ***snf5-related 1***. We find *snr1* to be an essential gene and that both the snr1 and brm proteins are part of a large complex. Our findings provide direct evidence that a relative of the yeast SWI/SNF complex is present in *Drosophila* and is involved in regulating the transcription of homeotic and other genes during development.

MATERIALS AND METHODS

Isolation of cDNA Clones and DNA Sequence Analysis

A 1-kb *ini1* partial cDNA fragment was labeled by random priming (Sambrook *et al.*, 1989) and hybridized to a *Drosophila* cDNA library

obtained from larval imaginal discs (Brown and Kafatos, 1988). Approximately 500,000 recombinants were screened using low stringency conditions. The filters were incubated for >18 h at 55°C in 5× SSPE, 5× Denhardt's, 200 μg/ml salmon sperm DNA, 0.5% sodium dodecyl sulfate (SDS), 10% dextran sulfate, and washed three times for 30 min at room temperature in 2× SSC, 0.5% SDS. The full DNA sequence on both strands was obtained using overlapping subclones and sequence-specific primers (Operon, Alameda, CA) either by the dideoxy procedure with the Sequenase kit (United States Biochemical, Cleveland, OH) or by automated sequencing on an Applied Biosystems apparatus (ABI, Columbia, MD). The *snr1* sequence was used to search the GenBank and EMBL databases for related genes by the FASTDB method (IntelliGenetics, Mountain View, CA). The National Center for Biotechnology Information BLAST electronic mail server was used to identify sequences related to *snr1* in the GenBank 86.0, EMBL 40.0, PIR 41.1, and SWISS-PROT 30.0 data bases, using the tblastn and blastp programs (Altschul *et al.*, 1990) and the BLOSUM62 matrix (Henikoff and Henikoff, 1992). Alignments were performed using the BESTFIT program (Wisconsin Genetics Computer Group) and Pustell matrix analysis (MacVector 4.1.1 software, IBI, New Haven, CT; Pustell and Kafatos, 1982). The *snr1* sequence has been deposited into the GenBank database (accession number U28485).

Pulsed-Field Gel Analyses

High molecular weight chromosomal DNA from several P-element *Drosophila* lines was prepared for pulsed-field gel analysis (D. Garza, personal communication). Frozen adult flies (100) of the appropriate genotype were ground to a fine powder with a mortar and pestle. The powder was mixed with 3 ml ice cold nuclear isolation buffer (NIB; 10 mM Tris, pH 8.5; 60 mM NaCl; 10 mM EDTA; 0.15 mM spermine; 0.15 mM spermidine; 0.5% Triton X-100) and dounce homogenized. The suspension was centrifuged at 3000 rpm in an IEC clinical centrifuge at 4°C for 15 s. The supernatant containing cell nuclei was removed to prechilled 1.5 ml microcentrifuge tubes and centrifuged at ~3000 rpm for 5 min at 4°C. The supernatant was removed and the nuclei pellet gently resuspended in 500 μl of NIB and centrifuged as before. The nuclei pellet was gently resuspended in 100 μl NIB and warmed briefly to 37°C, and then mixed with 150 μl of 1.2% low melting point agarose, 0.125 M EDTA. The mixture was poured into plug molds and allowed to harden at 4°C. Plugs were prepared for electrophoresis as described (Gemmill *et al.*, 1992). The chromosomal DNA was digested with either *Not*I or *Xba*I (Boehringer Mannheim, Indianapolis, IN), electrophoresed through an 0.8% agarose gel with an 8 s pulse time, then transferred to a Hybond-N (Amersham, Arlington Heights, IL) nylon filter. Hybridization was performed using standard conditions (Sambrook *et al.*, 1989).

Isolation of DNA, RNA, and Nucleic Acid Blot Analyses

Chromosomal *Drosophila* DNA isolated from the P-element excision lines was examined by polymerase chain reaction (PCR) analysis (Rasmusson *et al.*, 1993) using primers generated from the sequence of the *snr1* cDNA or from the terminal ends of the P-element (IR primer; Rasmusson *et al.*, 1993). Chromosomal DNA used for Southern blots of the P-element excision lines was prepared essentially as described by Roberts (1986). Hybridization of the *snr1* cDNA to genomic DNA blots was carried out as described above. RNA was isolated and analyzed by Northern blotting as described by Tamkun *et al.* (1992). The RNA blot was simultaneously hybridized with random-primed cDNA probes for both *snr1* and *brm*, using standard conditions.

Production of Antibodies, Western Blotting, and Immunostaining of Embryos

A 940-bp *Sac*II–*Eco*RI fragment of the *snr1* cDNA (nucleotide 439 to an *Eco*RI site in the polylinker) was cloned into the TrpE fusion vector pATH10 by addition of *Eco*RI linkers to the *Sac*II site. Induction and purification of inclusion bodies was performed as previously described (Carroll and Laughon, 1987). Rats were injected with 50 μg protein per boost using the Ribi Adjuvant System (Ribi). Whole anti-sera was used at a dilution of 1:250 to 1:500 for localization of the snr1 protein in *Drosophila* embryos and at a dilution of 1:450 or 1:500 for Western immunoblot analyses.

Extracts were prepared from staged Oregon-R embryos for Western analysis. Embryos were dechorionated, washed, and homogenized in (1:1 w/v) 1× sample buffer (2.5% SDS, 10% glycerol, 62.5 mM Tris, pH 6.8). Samples were then boiled for 2 min followed by microcentrifugation for 5 min at room temperature to pellet insoluble material. Samples were electrophoresed through 11% SDS-polyacrylamide gels as above, and transferred by electroblotting to nitrocellulose (Towbin *et al.*, 1979). Filters were blocked for 30 min at room temperature in 1× Tris buffered-saline (TBS; 100 mM Tris, pH7.5, 0.9% NaCl), with 10% nonfat dry milk, 3% BSA, and 4% normal goat serum. Incubation with rat anti-snr1 serum was carried out in blocking buffer (without milk) overnight at 4°C. The filters were washed in TTBS (TBS, 0.1% Tween 80) and incubated at room temperature for 30 min with goat anti-rat secondary antibody (Jackson Immuno Research Labs, West Grove, PA) conjugated to horseradish peroxidase at a dilution of 1:10,000. Filters were washed as above and developed with the enhanced chemiluminescence (ECL) method (Amersham).

Embryos used for whole mount antibody detection of snr1 protein were fixed and stained as described by Reuter *et al.* (1990). Antibody-stained embryos were viewed on a Zeiss Axiophot microscope (Thornwood, NY) with Nomarski optics and photographed on Kodak Ektachrome 64 Tungsten film (Rochester, NY).

Fly Strains and Genetic Manipulations

All fly strains were raised at 25°C, unless otherwise noted. The P-element enhancer trap lines, including AS1319, were cytologically mapped by T. Laverty (University of California, Berkeley, CA) and were generously provided as part of the *Drosophila* Genome Project. During the course of this work, we mapped the lethality of the P-element strain AS1319 to the *snr1* gene, and for this reason named this allele $snr1^{P1}$. Excision/transposition of the P-element in AS1319 was induced after the introduction of a stable source of transposase from *P[ry⁺ [Δ2,3](99B)* (Laski *et al.*, 1986). Twenty females of the genotype $snr1^{P1}$*/TM3, ry506* were mated with 20 males of the genotype *P[ry⁺ [Δ2,3](99B)/TM6B*. Male progeny (200) of the genotype $snr1^{P1}$*/P[ry⁺ [Δ2,3](99B)* were pair-mated to virgin females of the genotype *TM3, ry506/TM6B*, and ry^- progeny were selected. Eighty independent ry^- progeny were then analyzed by genetic complementation of the lethality associated with the $snr1^{P1}$ allele. These potential new alleles of *snr1* were also molecularly characterized by PCR and Southern blot analyses.

Interaction crosses between *snr1*, *brm*, and *trx* were carried out essentially as described (Kennison and Tamkun, 1988; Tamkun *et al.*, 1992), except that crosses were maintained at 23°C. The $snr1^{P1rev}$ stock is a viable excision line obtained as described above, that fully complements the lethality of both $snr1^{P1}$ and $snr1^{R3}$.

Superose 6 Chromatography

Nuclear proteins were obtained from *Drosophila* embryos as described by Kamakaka *et al.* (1991). The nuclear extract was applied to a Sepharose G25 column equilibrated in 50 mM sodium phosphate, pH 7.8, 425 mM NaCl and the excluded protein was concentrated to approximately 4 mg/ml. Eight hundred micrograms of this material was applied to a Superose 6 fast-performance liquid chromatography (FPLC) column, with elution of the protein in 50 mM

sodium phosphate, pH 7.8, 425 mM NaCl. brm and snr1 proteins in the 0.5-ml fractions were detected by immunoblotting as described above.

Epitope-tagging of the brm Protein and Immunoprecipitation Assays

A 14.4-kb *Bam*HI–*Eco*RI genomic DNA fragment spanning the *brm* gene (Brizuela *et al.*, 1994) was modified using PCR to create a brm transgene encoding a protein in which the C-terminal two residues of the brm are replaced by the sequence SSYPYDVPDYASSHHH-HHH. This tag contains the 9-amino acid epitope of the influenza hemagglutinin (HA) protein, which is recognized by the monoclonal antibody 12CA5(BAbCo). The modified fragment was subcloned into the P-element transformation vector CaSpeR and transformed into the germ line of *Df(1)w67c2, y* embryos as described previously. Five independent transgenic lines were generated and found to complement the recessive lethality of an extreme *brm* allele. A transgenic line (*Df(1)w67c2, y P[w$^+$ 9222–3 brm-HA]*) homozygous for an insertion of the transgene on the X chromosome was used for the studies described below.

Native protein extracts were prepared from either control (*Df(1)w67c2,y*) or transgenic (*Df(1)w67c2, y P[w$^+$ 9222–3 brm-HA]*) embryos as follows. Embryos (0–12 h) were dechorionated in 50% bleach for 2 min and washed extensively in 0.7% NaCl, 0.03% Triton X-100. Approximately 0.5 g embryos were homogenized in an equal or greater volume of 40 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.0, 350 mM NaCl, 0.1% Tween-20, 10% glycerol, 100 μg/ml phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 2 μg/ml aprotinin, 1 μg/ml pepstatin, and 1 mM benzamidine and centrifuged in a TLA45 Beckman microfuge rotor at 45,000 rpm 30 min. Avoiding the top lipid layer, the supernatant was transferred to new tubes and stored at −80°C. The 12CA5 monoclonal antibody recognizes the brm protein in transgenic, but not *Df(1)w67c2, y*, protein extracts by Western blotting.

brm and associated proteins were immunoprecipitated from total embryo extracts using the 12CA5 ascites fluid. Ascites fluid (20 μl) was incubated for 1 h at 4°C with approximately 50 μl of Protein A-Affi-prep beads (Bio-Rad, Richmond, CA) and 130 μl of IP buffer (10 mM HEPES, pH 8.0, 1 mM EDTA, 10% glycerol, 50 mM NaCl); unbound antibody was then removed by washing with IP buffer. Twenty-five microliters of antibody-adsorbed beads was added to 300 μg of embryonic protein extract, brought to 200 μl total volume with IP buffer, and incubated at 4°C with rocking for 2 h. After centrifugation and extensive washing with IP buffer, bound material was eluted with 100 mM glycine, pH 2.75, and neutralized with 1/20 volume of 1 M NaH_2PO_4, pH 8.0. Unbound and bound proteins were fractionated on a 8% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane with the addition of 0.1% SDS. The upper and lower halves of the Western blot were probed with antibodies against brm and snr1, respectively.

RESULTS

Molecular Cloning of snr1

A partial *ini1* cDNA encompassing the C-terminal two-thirds of the predicted *ini1* open reading frame was hybridized to *Drosophila* genomic DNA under conditions of low stringency. A single *Eco*RI restriction fragment hybridized to the *ini1* fragment (our unpublished results), suggesting that only one close relative of *ini1* is present in flies. To isolate cDNA clones corresponding to this gene, we screened ~500,000 cDNA clones from a larval imaginal disc library (Brown and Kafatos, 1988) with the *ini1* fragment. Eleven clones were isolated and analyzed; each contained an insert of approximately 1.4 kb. Hybridization of cDNA clones to RNA blots of poly(A$^+$) mRNA and total RNA revealed a single 1.4-kb transcript (see Figure 6A), indicating that the cDNA clones are near full-length. The full sequence on both strands was determined for one of the cDNA clones and partial sequence was obtained for four other clones. With the exception of small differences in the length of some of the 5′ ends, all the cDNAs appear to be identical by restriction endonuclease digestion.

The full nucleotide sequence obtained from overlapping clones (Figure 1) encompasses a 1.1-kb open reading frame encoding a 370-amino acid protein with a predicted molecular weight of 43 kDa. The predicted protein coding region beginning at the first AUG (nucleotide position 128) is preceded by a consensus CAAC sequence common among *Drosophila* genes (Cavener, 1987). Stop codons in all three potential reading frames upstream of the predicted initiation codon would prevent use of other upstream AUGs. A consensus polyadenylation signal is located +65 bp from the end of the open reading frame and −18 bp from the poly(A) tail.

Based on its similarity to the yeast *SNF5* gene, we have named this *Drosophila* gene *snr1* for ***snf5-related 1***. The predicted snr1 and ini1 proteins are similar in size and highly related over their entire lengths (78% similarity; 65% identity; Figures 2 and 3). In contrast, the snr1 and SNF5 proteins are only distantly related. The 370-residue snr1 protein is much shorter than the 904-residue SNF5 protein, due to the absence of the glutamine-rich and proline-rich segments found at the ends of the SNF5 protein (Figure 2). The glutamine-rich N-terminal region of SNF5 is not essential for SNF5 function (Laurent *et al.*, 1990). The most highly conserved region of snr1 and SNF5 (50% similarity; 41% identity) is a 200-amino acid acidic region including the entire C-terminus of snr1 (Figure 3). This region is also highly conserved between ini1 and snr1 (86% identical). The similarities between snr1, ini1, and SNF5 are restricted to a relatively short segment, suggesting that this region may represent a discrete functional domain. Outside this domain, the snr1 and SNF5 proteins are highly divergent.

A search of the available nucleic acid and protein data bases using both the FASTDB and BLAST programs revealed that snr1 is also significantly related to a *C. elegans* gene (CeSNF5), recently identified as part of the worm genome sequencing project (GenBank #Z32683). The deduced snr1 and CeSNF5 protein sequences are approximately 67% similar and 53% identical over their entire predicted lengths (Figure 4). snr1 is also distantly related to the yeast transcription elongation factor S-II, one of a group of yeast strand-transfer proteins. Although this similarity is intriguing in light of the interaction between HIV integrase and ini1, the resemblance is too limited to conclude that

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   1 GAATAGACGCCATGTACATGTCTGTGTTTGTGTATGCGCCAAGAAAATTTAAGTCGCCGGAATATTAACAAGGAATCCCGGCCAGAAACA   90

  91 GGGCATTGAGAGACCACAGAAGAAAATATACCCAACATGGCACTGCAGACATACGGGGACAAGCCGGTGGCCTTCCAGCTGGAGGAGGGC  180
   1                                   M  A  L  Q  T  Y  G  D  K  P  V  A  F  Q  L  E  E  G   18

 181 GGCGAGTACTACTACGTGGGCTCGGAAGTGGGCAACTACATGCGCCACTTCCGCGGCATTCTGTACAAGAAGTACCCGGGAATGACCCGC  270
  19  G  E  Y  Y  Y  V  G  S  E  V  G  N  Y  M  R  H  F  R  G  I  L  Y  K  K  Y  P  G  M  T  R   48

 271 ATCGTCCTGTCCAACGAGGAGCGCAAGCGGCTGGCTGAGTCCGGCCTCAGCTCCCACATCTTAGCCAGCTCTGTATCGCTGCTCCGCGCC  360
  49  I  V  L  S  N  E  E  R  K  R  L  A  E  S  G  L  S  S  H  I  L  A  S  S  V  S  L  L  R  A   78

 361 GTAGAGGTGGACGATATCATGGCCGGCAACGATGAAAAGTATCGCGCCGTCTCCGTGAACACTTCCGATACGCCAGTGCCGCGGGAGAGC  450
  79  V  E  V  D  D  I  M  A  G  N  D  E  K  Y  R  A  V  S  V  N  T  S  D  T  P  V  P  R  E  S  108

 451 AAGTCAAAGAAGCAGCCACAGTATGTGCCCACGATGCCGAACTCCAGCCACCTGGACGCAGTGCCACAGCCACGCCAATCAACCGAAACC  540
 109  K  S  K  K  Q  P  Q  Y  V  P  T  M  P  N  S  S  H  L  D  A  V  P  Q  P  R  Q  S  T  E  T  138

 541 GAGTGCACACGAAGAAGGTTCGCACATTCCCGATGTGTTTCGACGACACGGATCCCACGGCTAGCCTGGAGAATGCCGGCGCAGAAGGAG  630
 139  E  C  T  R  R  R  F  A  H  S  R  C  V  S  T  T  R  I  P  R  L  A  W  R  M  P  A  Q  K  E  168

 631 TGCCTGGTGCCCATTCGACTGGACATGGAGCTAGAGGGTCAAAAGCTGCGCGACACCTTCACGTGGAACAAGAACGAGAGCATGATTACT  720
 169  C  L  V  P  I  R  L  D  M  E  L  E  G  Q  K  L  R  D  T  F  T  W  N  K  N  E  S  M  I  T  198

 721 CCGGAGCAGTTTGCCGAGGTGCTGTGCGACGACCTGGACCTCAATCCCCTGCCCTTTGTGCCGGCTATTGCACAGGCCATCCGACAGCAG  810
 199  P  E  Q  F  A  E  V  L  C  D  D  L  D  L  N  P  L  P  F  V  P  A  I  A  Q  A  I  R  Q  Q  228

 811 ATCGAAGCCTTTCCCAACGATCCCCCCATCCTCGAGGAGACCTGCGACCAGCGGGTCATTGTTAAGCTGAACATTCACGTGGGCAACACC  900
 229  I  E  A  F  P  N  D  P  P  I  L  E  E  T  C  D  Q  R  V  I  V  K  L  N  I  H  V  G  N  T  258

 901 TCGCTCGTCGACCAGGTCGAGTGGGACATGTCCGAGAAGAACAACAACCCCGAGGAGTTTGCCATTAAACTCTGTGCGGAATTGGGATTG  990
 259  S  L  V  D  Q  V  E  W  D  M  S  E  K  N  N  N  P  E  E  F  A  I  K  L  C  A  E  L  G  L  288

 991 GGAGGAGAGTTTGTTACGGCCATTGCCTACAGCATTAGGGGTCAGCTATCGTGGCACTGTCGAACGTACGCCTTCAGCGAGGCCCCTCTA 1080
 289  G  G  E  F  V  T  A  I  A  Y  S  I  R  G  Q  L  S  W  H  C  R  T  Y  A  F  S  E  A  P  L  318

1081 TCAACGATTGATGTGCCCTTCCGGAATCCCAGCGACGCTGACGCATGGGCGCCATTCCTAGAGACGCTTACCGACGCCGAAATGGAGAAG 1170
 319  S  T  I  D  V  P  F  R  N  P  S  D  A  D  A  W  A  P  F  L  E  T  L  T  D  A  E  M  E  K  348

1171 AAAATCCGCGACCAGGACCGCAACACGCGCAGAATGCGACGACTGGCCAATACCACAACTGGTTGGTGATCTTCCGCCGATCCAGCAATG 1260
 349  K  I  R  D  Q  D  R  N  T  R  R  M  R  R  L  A  N  T  T  T  G  W  *                        370

1261 TGTCACTAATGTAATCCTCCTATTAAGTACCATTATGCATCCCAAATAAAGTTGTGTCTGTCGATTTTAAAAAAAAAAAAAAAAAA 1345
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Figure 1. Nucleotide and predicted amino acid sequence of the *snr1* gene. The nucleotide sequence of the longest cDNA is shown. The single predicted open reading frame of 1.1 kb could encode a 370-amino acid protein of approximately 43 kDa, beginning with the ATG located at nucleotide position 127 and ending at position 1239. A consensus polyadenylation signal (shown in bold) is located +65 bp from the end of the open reading frame and −18 bp from the poly(A) tail.

S-II is functionally related to either snr1 or ini1. In contrast, the fly, worm, and human SNF5-related proteins are strikingly similar over their entire lengths, which suggests they are functional homologues.

We also examined the possibility that *snr1*, like *brm* and *SWI2/SNF2*, is a member of a gene family. Hybridization of both *snr1* and *ini1* cDNAs to *Drosophila* genomic restriction fragments revealed no obvious additional fly genes. Low stringency hybridization of the yeast *SNF5* gene to yeast genomic DNA also does not reveal other related gene(s) (Dingwall and Scott, unpublished observations). Thus in contrast to the *SWI2/SNF2* family (Carlson and Laurent, 1994; Elfring *et al.*, 1994) no evidence has been obtained for a family of genes closely related to *SNF5* in either *Drosophila* or yeast. Although *snr1* appears to be the only *Drosophila* gene closely related to *SNF5*, the sequence similarity be-

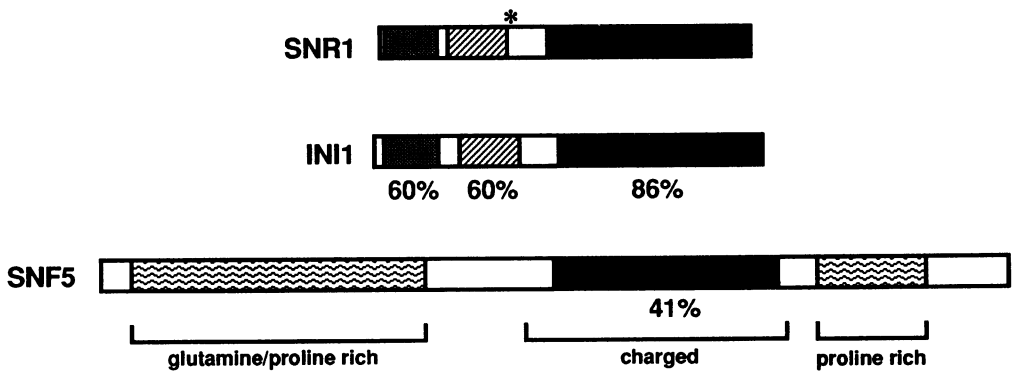

Figure 2. The snr1 and ini1 proteins are related to the yeast SNF5 protein. The snr1, ini1, and SNF5 proteins are shown in diagrammatic form highlighting regions of strongest similarity. The predicted snr1 and ini1 proteins show 65% overall homology, with three subregions that vary from 60% to 86% identity. The region of strongest identity (black box) is sufficient for ini1 interaction with HIV integrase. Both snr1 and ini1 also contain highly charged regions, like SNF5, with a strongly acidic core (−13 charge over 98 amino acids, aa 194–291 of the snr1 sequence). The charged regions of snr1 and ini1 show the highest similarity to SNF5 (41% identity over 200 amino acids). SNF5 contains two regions not found in snr1 or ini1, including a large nonessential polyglutamine region at the N-terminus and a proline-rich region near the C-terminus. The asterisk above the snr1 diagram indicates the position of a lethal P-element insertion within the snr1 gene (aa 131).

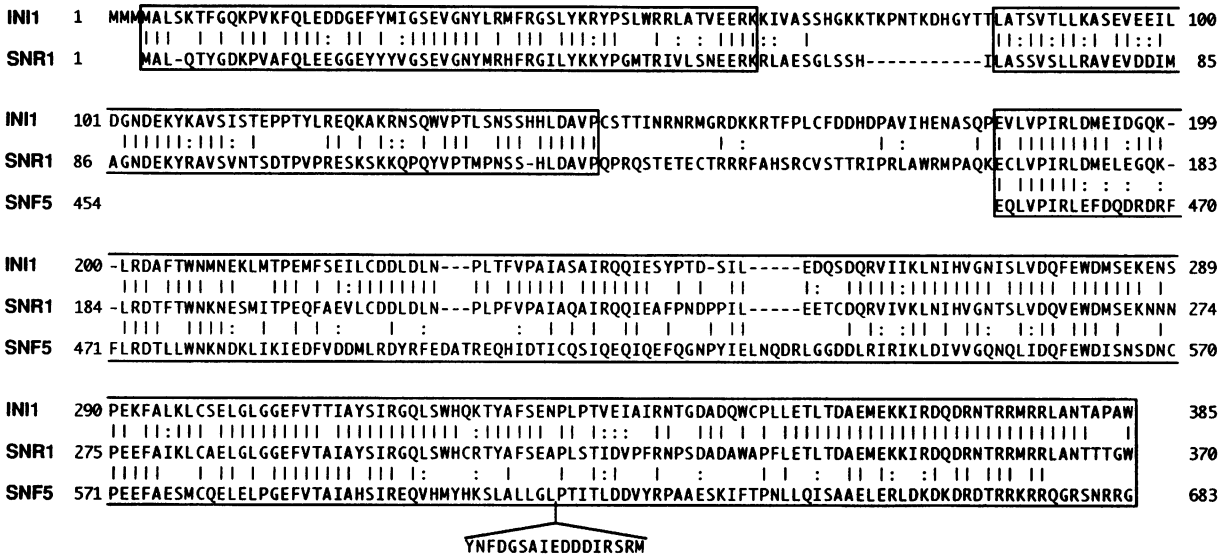

Figure 3. Sequence similarities among SNF5-related proteins. A direct sequence alignment of snr1, ini1, and SNF5 reveals strong conservation. The full length snr1 and ini1 deduced protein sequences are shown, whereas only the region of highest similarity to SNF5 is presented. The ini1 protein contains an 11-amino acid stretch not found in snr1 or SNF5, indicated by the gap between the two 60% identity regions (aa 72 to aa 82); furthermore, this region was not present within some of the ini1 clones sequenced (Kalpana *et al.*, 1994), suggesting that it may be either an exon unique to ini1 or that it was included in some cDNA clones as a result of alternate or incomplete processing. The snr1 and ini1 proteins are also nearly co-linear with a 200-amino acid portion of SNF5, with the exception of a 17-amino acid stretch. Outside of this region, there is little conservation between the yeast and fly proteins. The snr1 protein is truncated at Gln 131 in both the $snr1^{P1}$ insertion mutant and in the $snr1^{R3}$ lethal excision mutant.

tween the snr1 and SNF5 proteins is too limited to conclude that they are functional homologues.

Genetic Analysis of snr1 Mutants

In situ hybridization of the *snr1* cDNA to salivary gland polytene chromosomes locates *snr1* near the base of the right arm of the third chromosome, at cytological position 83A5,6. With the exception of the small subunit of RNA polymerase, no known genes or mutations have been mapped to this region, nor are deficiencies available. A screen for dominant modifiers of homeotic mutations identified a number of previously uncharacterized genes including *brm* (Kennison and Tamkun, 1988), but none map to 83A. Thus, *snr1* does not appear to correspond to any previously known gene.

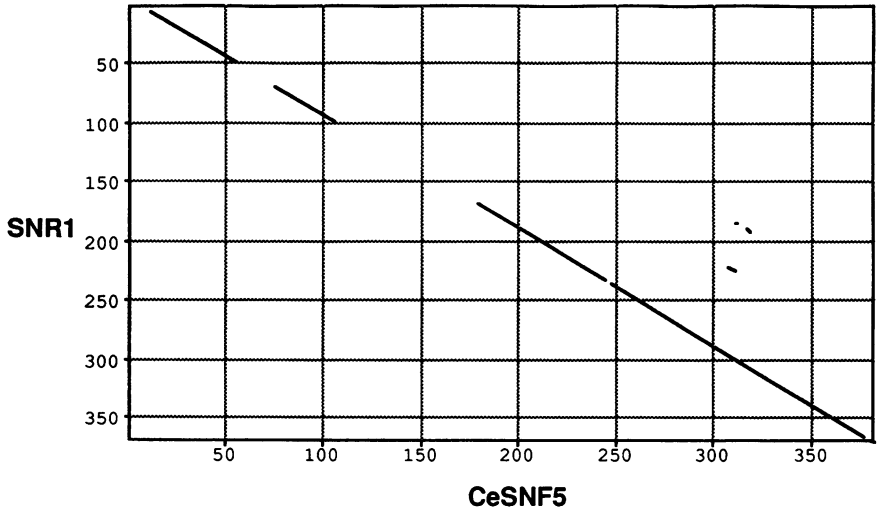

Figure 4. Matrix alignment of snr1 with a *Caenorhabditis elegans* SNF5-related protein. A *C. elegans* genomic sequence is predicted to encode a protein of 382 amino acids and shows a strong similarity to the snr1 protein using a Pustell protein matrix (MacVector software, International Biotechnologies). The PAM250 scoring matrix (Pearson, 1990), window size of 15 residues, and a minimum score of 35% was used in the analysis. The alignment indicates that the predicted proteins are nearly co-linear, with an overall similarity of 53% identity. The highest identity between the two proteins is within the 200-amino acid region conserved among all four SNF5-related proteins. The gap in the alignment roughly corresponds to the same region poorly conserved between the fly and human proteins.

To begin a genetic analysis of the *snr1* gene we examined lethal ry^+ P-element transposon insertions located in the vicinity of 83A (*Drosophila* Genome Project, University of California, Berkeley, CA). Pulsed field gel electrophoresis was used to map four of these lethal insertions relative to the *snr1* gene (our unpublished results). The *snr1* gene is contained within a 250-kb *Not*I restriction fragment (Figure 5). One of the insertions, in the fly stock AS1319, has a restriction fragment polymorphism within the 240-kb fragment detected with the *snr1* cDNA. Additional restriction enzyme analysis, in combination with PCR using P-element–specific and *snr1*-specific primers, indicates that the AS1319 insertion is located within the *snr1* gene (Figure 5). Genomic sequences flanking the insertion site were obtained by PCR and by the plasmid-rescue technique (Bier *et al.*, 1989). Sequencing revealed that the P-element insertion in AS1319 had occurred within an exon of *snr1* (Figure 5). The *snr1* gene is transcribed in a centromere proximal to distal direction.

The mobilization of the P-element allowed construction of additional mutations by transposon excision (Cooley *et al.*, 1988). A source of P-transposase was introduced and progeny were scored for loss of the ry^+ marker contained within the P-element. One hundred four ry^- progeny were obtained and 80 of these lines were analyzed using a combination of PCR, Southern blot analysis, and genetic complementation of the lethality of the AS1319 mutant. In every case, ry^- excision lines that retained portions of the P-element, due to incomplete or imprecise excision, failed to complement the lethality associated with AS1319 (32/80 lines). Twenty-six of the 80 ry^- lines tested had no remaining P-element sequences and all complemented the lethality of AS1319. This lethality was thus due to an insertion within the *snr1* gene, so we named the allele $snr1^{P1}$. We therefore conclude that *snr1* is essential for viability.

The remaining 22 excision lines potentially represent new alleles of *snr1*, because each excision chromosome failed to complement the AS1319 lethality and contained no P-element sequences. Chromosomal DNA surrounding the original insertion site was cloned from two lines ($snr1^{R3}$ and $snr1^{R10}$) using PCR primers specific to *snr1*. In both cases, imprecise excision had generated in-frame translation termination codons at amino acid 131. The truncations effectively eliminate the C-terminal two-thirds of the protein, including the regions of highest sequence similarity between the snr1, SNF5, and ini1 proteins (Figure 2). PCR and DNA blot analyses did not reveal any significant deletions of surrounding chromosomal DNA in the 22 lethal excision lines.

Both $snr1^{P1}$ and $snr1^{R3}$ mutant homozygotes die during the larval period of development before the

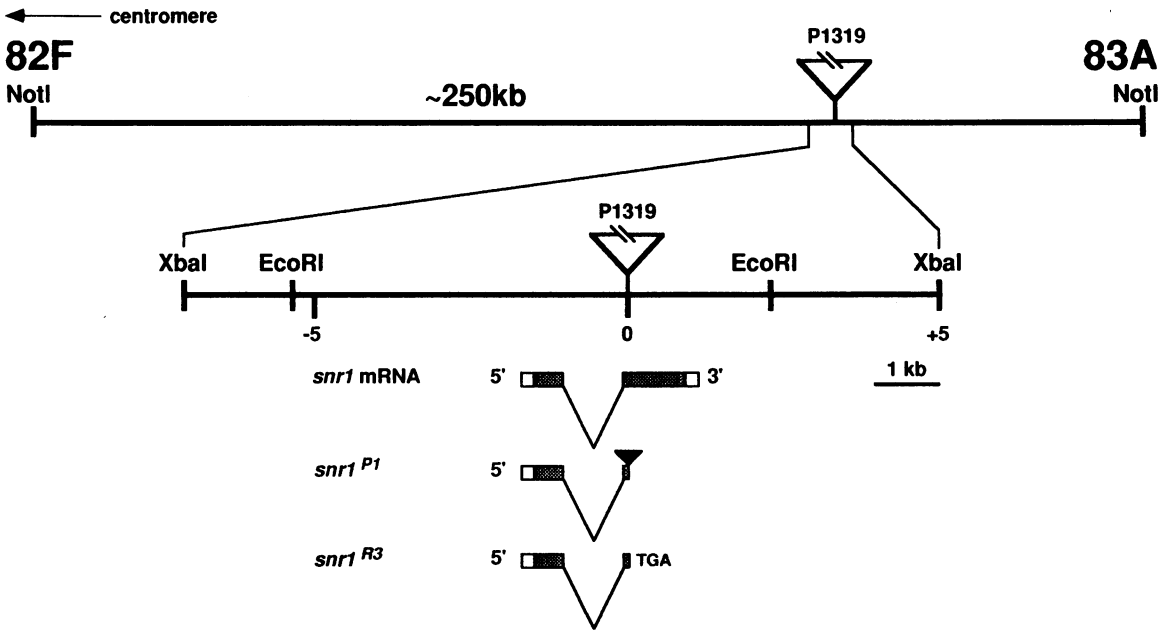

Figure 5. Molecular map of the *snr1* region. Proximal is to the left and distal is to the right. The *snr1* cDNA localizes to the salivary gland polytene chromosome region 83A5,6. A lethal P-element insertion (P1319) was localized within a 250-kb *Not*I fragment and a 12-kb *Xba*I fragment by pulsed field gel electrophoresis. *Eco*RI sites within the 12-kb *Xba*I fragment are shown. The position of the *snr1* transcript relative to the insertion is shown in the lower half of the diagram. The site of the P-element insertion was chosen as the (0) position within the map. The shaded region of the *snr1* mRNA represents the open reading frame that encodes the snr1 protein. The $snr1^{P1}$ and $snr1^{R3}$ alleles (see text for details) are indicated below the molecular map.

third larval instar stage. No homeotic transformations or other phenotypes are seen in homozygotes, nor are distributions of several homeotic gene products including Ultrabithorax (Ubx) and Antennapedia (Antp) notably altered (our unpublished results). As discussed below, the lack of such phenotypes could be due to a large maternal contribution of *snr1* gene products.

To investigate a possible role for *snr1* in regulating homeotic gene transcription, we examined whether mutations in *snr1*, like *brm* mutations, suppress mutations in *Polycomb* and enhance the adult phenotypes of *trx* mutations. Heterozygous *brm* mutations and deficiencies strongly suppress the transformations seen in heterozygous *Polycomb* adults by preventing the derepression of homeotic genes (Kennison and Tamkun, 1988; Tamkun *et al.*, 1992). In contrast, the loss of one copy of *snr1* does not suppress adult *Polycomb* mutant phenotypes, such as transformations of second and third legs to first leg, wing to haltere, and abdominal segments to more posterior identities. The *snr1* product thus does not appear to be limiting under these assay conditions.

We also examined whether *snr1* interacts with trithorax group members, including *brm* and *trx*. *trx* encodes an activator of homeotic gene transcription (Mazo *et al.*, 1990; Breen and Harte, 1991) and heterozygous mutant adults sometimes display homeotic transformations of thoracic and abdominal segments due to the decreased expression of ANT-C and BX-C genes (Lewis, 1968; Ingham and Whittle, 1980; Ingham, 1983). Heterozygous mutations in several trithorax group genes, including *brm*, enhance *trx* mutant phenotypes, such as the anterior transformation of the fifth abdominal segment (A5) (Shearn, 1989; Tamkun *et al.*, 1992). A *snr1* mutation also enhances the abdominal transformations seen in *trx* heterozygotes (Table 1). Individuals containing mutations in all three genes (*snr1*, *brm*, and *trx*) have even stronger transformations (Table 1). As a control, we used a chromosome from which the lethal P-element insertion in $snr1^{P1}$ had been excised ($snr1^{P1rev}$) and fully complemented a *snr1* mutation. In contrast to the *snr1* mutant, the $snr1^{P1rev}$ chromosome does not interact with *trx*.

snr1 and *brm* also interact genetically. Individuals heterozygous for either *snr1* or *brm* mutations are phenotypically wild type (Tamkun *et al.*, 1992; our unpublished results). In contrast, approximately 10% of *snr1/brm* transheterozygous adults display prothoracic defects, including the loss of the humerus. This phenotype is similar to that resulting from decreased function of brm (Tamkun *et al.*, 1992; Brizuela *et al.*, 1994) or Antennapedia (Abbott and Kaufman, 1986) during larval development. These genetic interactions suggest that *snr1* and *brm* act together, and with *trx*, to regulate homeotic gene transcription.

snr1 Expression during Development

To further explore the function of *snr1*, we characterized its temporal and spatial expression during development. An RNA blot containing poly(A)$^+$ mRNA from different embryonic stages was probed simultaneously with cDNAs for both *snr1* and *brm* (Figure 6A). The *snr1* mRNA appears as a 1.4-kb band, whereas the *brm* mRNA appears as a 5.5-kb band (Tamkun *et al.*, 1992). The timing and variation in level of the *brm* and *snr1* mRNAs are similar, although not identical. The highest level of mRNA accumulation for both genes occurs in unfertilized eggs and early embryos, indicating maternal contributions of both mRNAs. The mRNA accumulation levels decrease steadily throughout embryogenesis until approximately 16 h post-fertilization, when levels dramatically decrease (Figure 6A). By the end of embryogenesis (16–24 h) little *snr1* or *brm* mRNA is detectable. A low level of mRNA accumulates during larval and pupal stages but little, if any, RNA is found in adult males. Therefore, *snr1* is unlikely to provide an essential function to all cells.

A rat polyclonal antiserum was generated against the C-terminal two-thirds of the snr1 protein to examine the developmental accumulation and tissue distribution of the protein. The antibodies were tested for

Table 1. Interactions of *snr1* with *brm* and *trx*

Genotype	No. males scored	A5 transformation*		
		none	weak	strong
$nsr1^{P1rev}/trx^{E2}$	119	83 (70%)	36 (30%)	0
$snr1^{R3}/trx^{E2}$	98	36 (37%)	57 (58%)	5 (5%)
$snr1^{P1rev}/brm^{2}trx^{E2}$	121	53 (43%)	54 (45%)	14 (12%)
$snr1^{R3}/brm^{2}trx^{E2}$	94	2 (2%)	43 (46%)	49 (52%)

*Homeotic transformations were scored as the transformation of the A5 abdominal segment into the identity of the A4 segment, indicated by the loss of pigment from the A5 segment. Transformations were considered to be strong if greater than half the segment lacked pigmentation. The penetrance for each genotype is expressed as a percentage of males showing transformations.

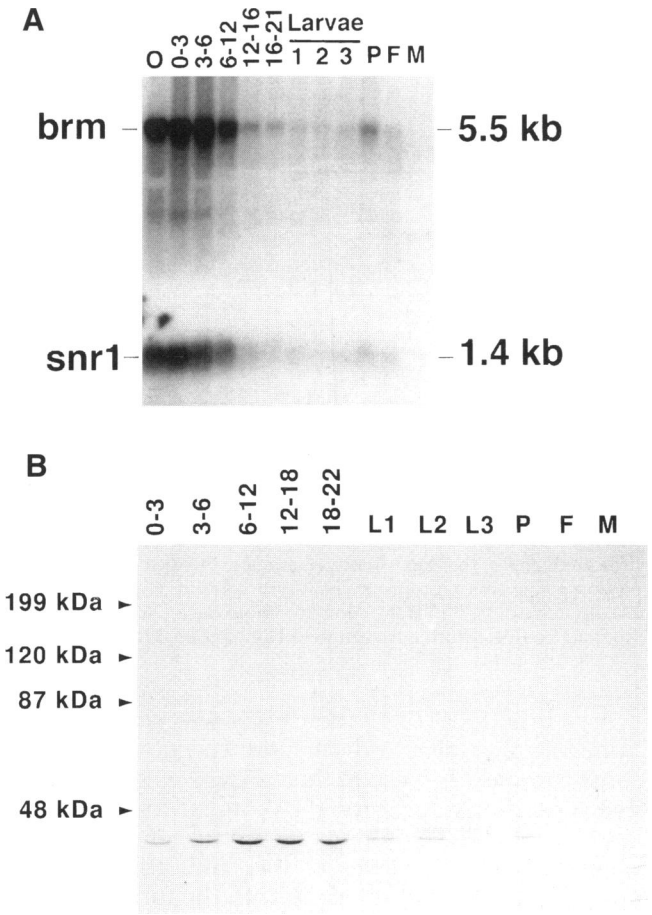

Figure 6. Developmental expression of *snr1* mRNA and protein. (A) A blot containing RNA isolated from oocytes (O), embryos (0–3, 3–6, 6–12, 12–16, and 16–21 h) larvae (L1, L2, and L3), pupae (P), and adult females (F) and males (M) was probed with random-primed probes for both the *snr1* and *brm* (cDNA 1′) cDNAs (Tamkun *et al.*, 1992). The blot was washed under high stringency conditions. The 1.4-kb *snr1* and the 5.5-kb *brm* transcripts are indicated. (B) Developmental expression of the snr1 protein. Extracts prepared from embryos, larvae, pupae, and adults (50 μg/lane) were electrophoresed on 12% polyacrylamide/SDS gels, blotted onto nitrocellulose, and incubated with a rat polyclonal antibody to the snr1 protein at a 1:450 dilution. After incubation with secondary antibody, the snr1 protein (43 kDa) was detected using the Amersham ECL chemiluminescence kit.

specificity using protein blots of several bacterially expressed snr1 fusions and by testing both embryos and protein blots of embryonic extracts with pre-immune serum (our unpublished results). Extracts from developmentally staged wild-type embryos, larvae, pupae, and adults were probed with the snr1-specific antisera (Figure 6B). The snr1 protein appears as a 43-kDa band, consistent with the size predicted from the *snr1* cDNA sequence and Northern blot analysis. The amount of snr1 protein peaks early in embryogenesis with low levels found throughout larval and pupal development. No snr1 protein is detected in adult males, consistent with the RNA accumulation data.

The distribution of snr1 protein during embryogenesis was determined by whole mount staining with the snr1 antibody (Figure 7). In agreement with the RNA and protein analyses presented above, snr1 protein is detected at the earliest stages of development. The protein is clearly associated with nuclei before cellularization (Figure 7A). Consistent with the localization of yeast SNF5 (Laurent *et al.*, 1990), the snr1 protein is located in the nucleus throughout embryogenesis. The snr1 protein is found in all nuclei of the embryo through the germ band extended stage (Figure 7B). The snr1 protein is located almost exclusively in the central nervous system and brain after retraction of the germ band (Figure 7, C and D). *snr1* mRNA is similarly distributed during embryogenesis as determined by in situ hybridization (our unpublished results). The imaginal discs and salivary glands of larvae have a uniform nuclear distribution of the snr1 protein, but there is no observable protein in other tissues (our unpublished results).

The temporal and spatial expression pattern of *snr1* mRNA is similar to that observed for *brm* (Tamkun *et al.*, 1992; Elfring *et al.*, 1994), consistent with their genetic cooperation in regulating homeotic gene transcription. In contrast to the homeotic proteins, which are produced in discrete domains along the anterior-posterior axis, *snr1* and *brm* products are fairly uniformly distributed along the embryo. The spatially and temporally restricted patterns of *snr1* expression, like *brm*, argue against a general role for *snr1* in transcription or other cellular processes.

A High Molecular Weight Complex Contains the snr1 and brm Proteins

The sequence similarity between snr1 and SNF5 suggests that snr1 might also function in concert with other proteins as part of a *Drosophila* counterpart of the yeast SWI/SNF complex. To test this possibility, we determined whether snr1 and brm are present in high molecular weight complexes. A soluble nuclear extract from 0–12 h embryos was prepared and fractionated on a Superose 6 FPLC column in moderate strength ionic buffer (425 mM NaCl). Under denaturing conditions, the observed molecular weights of the snr1 and brm proteins are similar to those predicted from their sequence (43 kDa and 185 kDa, respectively). In contrast, under nondenaturing conditions, both snr1 and brm proteins elute from the gel filtration column with an apparent molecular mass of approximately 2×10^6 daltons (Figure 8). Little, if any, brm or snr1 protein elutes at the position of their deduced monomeric sizes, suggesting that all of the brm and snr1 protein in embryonic extracts is present in a high molecular mass complex(es). The apparent molecular mass for both

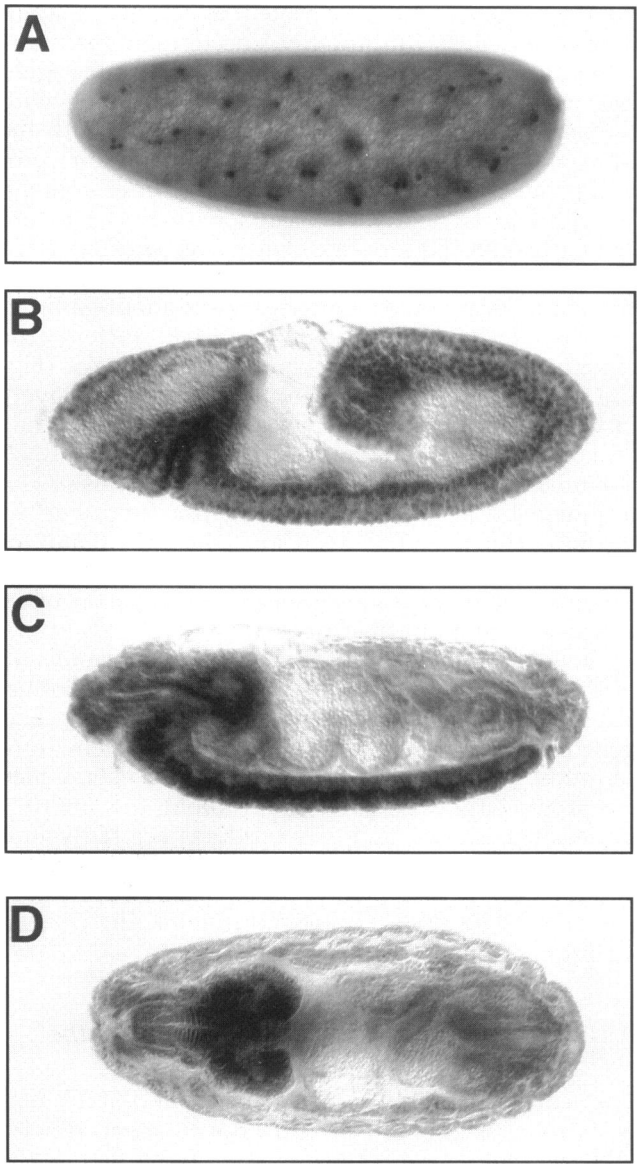

Figure 7. Embryonic expression of the snr1 protein. Embryos are oriented with anterior to the left. Wild-type Canton-S embryos were fixed and incubated with rat polyclonal sera against snr1 at a 1:500 dilution. (A) The snr1 protein localizes to nuclei early in development. The snr1 protein shows uniform distribution in all nuclei at the syncytial blasoderm stage (stage 3). (B) Stage 12 embryo at the start of germ band retraction. Most cells of the epidermis still express the snr1 protein, whereas the yolk cell nuclei do not. (C) Stage 15 embryo showing snr1 protein localized primarily to the central nervous system and brain. (D) Dorsal view of a stage 15 embryo showing high expression of snr1 in the brain. The staging is according to Campos-Ortega and Hartenstein (1985).

proteins is in close agreement with that observed for the yeast SWI/SNF complex (Peterson *et al.*, 1994) and for the human brg1 protein (Khavari *et al.*, 1993; Kwon *et al.*, 1994).

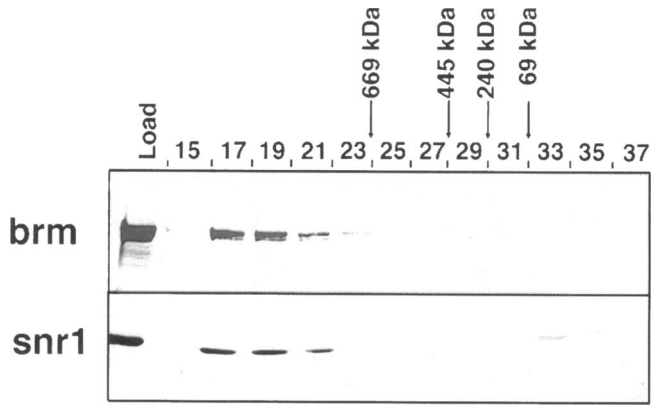

Figure 8. snr1 and brm are present together in large molecular mass complexes. snr1 and brm co-elute during chromatography on a gel filtration column. Embryonic nuclear extracts were fractionated on a FPLC Superose 6 column, and fractions were immunoblotted for detection of snr1 and brm proteins. Fraction numbers are indicated along the top. Arrows indicate the peak fractions for elution of the calibration proteins: thyroglobulin (669 kDa; fraction 24), apoferritin (445 kDa; fraction 28), B-amylase (240 kDa; fraction 30), and bovine serum albumin (69 kDa; fraction 32).

A co-immunoprecipitation assay was used to determine whether brm and snr1 are components of the same complex. For use in this assay, we constructed a gene encoding an epitope (HA)-tagged version of the brm protein. The epitope-tagged transgene fully complements extreme alleles of *brm*, indicating that the epitope tag does not interfere with the function of the brm protein (our unpublished results). Extracts from either transgenic (*Df(1)w67c2 y P[w$^+$ 9222–3 brm-HA]*) or control (*Df(1)w67c2, y*) embryos were incubated with a monoclonal antibody directed against the HA epitope and antibody-protein complexes were isolated using Protein A-coated beads. Bound proteins were eluted and analyzed by Western blotting. Neither brm nor snr1 protein bound to the anti-HA monoclonal antibody in extracts prepared from control embryos (Figure 9). In contrast, the anti-HA monoclonal antibody bound both brm and snr1 in extracts prepared from transgenic embryos (Figure 9). These results indicate that brm and snr1 are physically associated in embryonic extracts. Identical results were obtained when ethidium bromide (50 μg/ml) was added to extracts to alleviate protein-DNA interactions (Lai and Herr, 1992), indicating that the interaction between snr1 and brm is not due to indirect interactions via DNA (our unpublished results). Thus, like their yeast counterparts, the *Drosophila* snr1 and brm proteins are members of a huge protein complex.

DISCUSSION

The discovery of brm and snr1 in the same large protein complex in *Drosophila* provides strong evi-

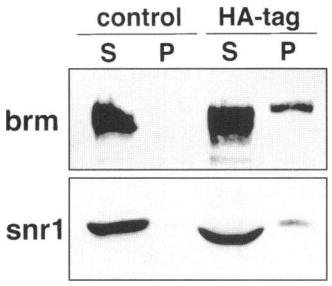

Figure 9. snr1 and brm co-immunoprecipitate. Embryonic extracts from a control (*Df(1)w67c2, y*) or a Brm-HA transgenic strain (*Df(1)w67c2, y P[w+ 9222–3 brm-HA]*) were incubated with a monoclonal antibody specific for the HA epitope and precipitated with Protein A-Sepharose beads. After precipitation and elution with glycine, the presence of both brm and snr1 was examined in the supernatant (S) and in the pelleted material eluted from the beads (P) by immunoblotting.

dence for conservation of a SWI/SNF-like complex from yeast to animals. The yeast SWI/SNF complex is required for the transcriptional induction of a diversely regulated set of yeast genes (Winston and Carlson, 1992; Carlson and Laurent, 1994). Genetic and biochemical studies suggest that the SWI/SNF complex is targeted to promoters via interactions with DNA-binding regulatory proteins, where it uses the energy of ATP hydrolysis to overcome the repressive effects of chromatin components, including nucleosomal histones, on transcription (Winston and Carlson, 1992; Cote *et al.*, 1994). The discovery that *brm*, an activator of *Drosophila* homeotic genes, is related to the yeast *SWI2/SNF2* gene provided an initial insight into molecular mechanisms underlying the action of Polycomb and trithorax group genes. Based on the structural and functional similarities between brm and SWI2/SNF2, one possibility is that brm, together with *Drosophila* homologues of other yeast SWI/SNF proteins, activates ANT-C and BX-C genes by overcoming the repressive effects of Polycomb group proteins (or other chromatin components) on transcription.

A Counterpart of the Yeast SWI/SNF Complex Is Present in Drosophila

A large number of *SWI2/SNF2* and *brm*-related genes have been identified in both mice and humans (reviewed in Carlson and Laurent, 1994), making it difficult to determine which, if any, of the vertebrate relatives are part of a SWI/SNF-like complex. Based on sequence homology, at least two human genes, *brg1* and *hbrm*, are closely related to *brm* and to each other (Khavari *et al.*, 1993; Muchardt and Yaniv, 1993). brg1 and hbrm are each capable of stimulating transcription, suggesting that they may be functional homologues of *brm*; brg1 is present in a large complex as well (Khavari *et al.*, 1993). An attempt to identify a human brg1 complex yielded two partially purified complexes (Kwon *et al.*, 1994) that potentially are counterparts to the yeast SWI/SNF complex (Imbalanzo *et al.*, 1994; Kwon *et al.*, 1994). The subunit compositions of these complexes have not been examined, so their relationships to the yeast complex are presently unclear. The existence of multiple human genes with sequences related to SWI2/SNF2 brings up the possibility of multiple complexes that may or may not be related to the yeast complex.

We searched for *Drosophila* relatives of other subunits of the yeast SWI/SNF complex and identified *snr1*, a distant relative of the yeast *SNF5* gene. Although SNF5 is an essential component of the yeast SWI/SNF complex (Laurent *et al.*, 1990; Peterson *et al.*, 1994), the biochemical function of the SNF5 protein is unknown. Like the SWI2/SNF2 and SNF5 proteins, brm and snr1 are members of huge ($\sim 2 \times 10^6$ Da) protein complexes. Using a co-immunoprecipitation assay, we found that the brm and snr1 proteins interact, either directly or indirectly. These data strongly suggest that snr1 and brm are members of a *Drosophila* counterpart of the yeast SWI/SNF complex. Although the exact composition of this *Drosophila* complex is unknown, it seems likely that it contains relatives of other subunits of the SWI/SNF complex, including SWI1, SWI3, and SNF6.

The existence of a brm/snr1 *Drosophila* complex related to the yeast SWI/SNF complex argues for both conservation of function and subunit composition of the complex during evolution. The retention of a relationship between two proteins in a large complex for about a billion years raises many questions, including the following: What functions of the complexes might be common to yeast and fly cells? How have the complexes changed to fulfill requirements specific to a higher eucaryote? What are the molecular mechanisms of complex function? Indeed, the existence of multiple SWI2/SNF2-related proteins in yeast and higher eucaryotes (reviewed in Carlson and Laurent, 1994; Peterson and Tamkun, 1995) and the differences between the SNF5 and snr1 sequences raise questions about the extent to which the properties of the SWI/SNF complex may be extrapolated to the brm/snr1 complex we detect in flies.

Roles of the brm/snr1 Complex during Drosophila Development

The temporal and spatial patterns of transcription of *snr1* products set limits on the gene's functions. *snr1* RNA and protein are present at highest levels early in embryogenesis and at relatively low levels in larvae and pupae. Neither *snr1* RNA or protein is expressed at detectable levels in adult males. *snr1* is expressed uniformly early in embryogenesis; in later embryos *snr1* RNA and protein is restricted to the central nervous system and brain. There is an approximate correlation between the occurrence of cell division and the expression of snr1; cell divisions cease in most cell types midway through embryogenesis, except in the

nervous system. Cell division occurs at high rates in imaginal discs, where snr1 products are also detectable.

Four specific conclusions can be drawn from the spatial and temporal patterns of snr1 expression. First, consistent with the results of our biochemical studies, snr1 and brm are expressed in similar spatial and temporal patterns during development. Second, both snr1 and brm are expressed throughout development at high levels in all cells where homeotic genes are actively transcribed. Third, the differential transcription of homeotic genes does not result from the differential expression of snr1 and brm; both snr1 and brm are expressed uniformly along the anterior-posterior axis at all developmental stages. Fourth, the restricted embryonic expression patterns of snr1 and brm, plus the absence of detectable levels of either mRNA or protein in adult males, implies that snr1 and brm are not required for all transcriptional activation.

What are the roles of *snr1* during *Drosophila* development? A *snr1* mutation strongly enhances the anterior transformation of the fifth abdominal segment seen in *trx* heterozygotes. The transformation is thought to be due to lowered activation of the BX-C homeotic genes by *trx* and, apparently, *snr1* (Ingham, 1983; Breen and Harte, 1993). The genetic interactions between *snr1*, *brm*, and *trx*, together with the physical association of the snr1 and brm proteins, defines *snr1* as a new member of the trithorax group of homeotic gene activators. *snr1* homozygotes die as second instar larvae with no discernable pattern defects or homeotic transformations. The lack of pattern defects in *snr1* mutant homozygotes is probably due to the high maternal contribution of *snr1* gene products. Like *snr1*, *brm* is expressed both maternally and zygotically. Individuals lacking zygotic *brm* activity die as unhatched larvae with no obvious pattern defects. Loss of maternal *brm* activity blocks oogenesis (Brizuela *et al.*, 1994). The brm/snr1 complex is therefore likely to play an important role in early development. We also anticipate that *snr1*, like *brm*, may be required for the activation of a large number of *Drosophila* genes. Conditional or dominant-negative mutations will be required to elucidate the roles of *snr1* and *brm* in oogenesis and embryogenesis.

Models for Polycomb Group and brm/snr1 Complex Functions in Light of SWI/SNF Mechanisms

What is the role of the brm/snr1 complex in homeotic gene regulation? DNA-binding regulatory proteins encoded by segmentation genes define the initial patterns of homeotic gene transcription relatively early in embryogenesis (for review see Harding and Levine, 1988; Ingham, 1988). The maintenance and refinement of these patterns depends on cross-regulatory interactions between homeotic genes, trithorax group genes, and Polycomb group genes. Current models favor the view that the Polycomb group of proteins silence transcription by compacting local regions of chromatin, rendering them inaccessible to the transcription machinery (Paro, 1993; Rastelli *et al.*, 1993). Polycomb complexes containing at least three products of Polycomb group genes (Rastelli *et al.*, 1993) are thought to assemble at specific transcription enhancer elements by interacting with segmentation proteins, such as hunchback, thus defining the transition from establishment to maintenance (Zhang and Bienz, 1992). However, because initiation and maintenance elements are in some cases physically separable, Polycomb group proteins may recognize a specific maintenance element (PRE or Polycomb Response Element; Simon *et al.*, 1993) through associations with an unidentified sequence-specific factor. None of the known Polycomb group proteins exhibit sequence-specific DNA binding, but polyhomeotic, Su(z)2 and Psc proteins contain potential zinc finger–like motifs and bind DNA nonspecifically in vitro (DeCamillis *et al.*, 1992; Rastelli *et al.*, 1993). The PRE site may act as a nucleation center to recruit additional Polycomb group proteins, which spread out along the chromosome and render genes transcriptionally inactive (Paro, 1993); therefore, the inactive state is heritable through cell divisions.

The trithorax group proteins, or some of them, may block assembly or function of Polycomb group complexes. Support for this model comes from experiments in yeast, where the SWI/SNF complex affects the association of histones with DNA (Hirschhorn *et al.*, 1992; Cote *et al.*, 1994) thereby "opening" chromatin to allow for enhanced binding by activators. The brm/snr1 complex might, by analogy to the yeast SWI/SNF complex, use the energy of ATP hydrolysis to counteract the repressive effects of Polycomb or other chromatin components on the transcription of homeotic genes by creating and/or sustaining a permissive chromatin environment for activators such as trx.

The brm/snr1 complex may be targeted to ANT-C and BX-C genes via interactions with either segmentation gene products or trx, which is thought to bind DNA directly (Kuzin *et al.*, 1994). The product of a segmentation gene, *fushi tarazu*, requires the SWI/SNF complex to activate transcription in yeast (Peterson and Herskowitz, 1992). Because of the strong genetic interactions between *trx*, *brm*, and *snr1*, the trx protein is a likely candidate for a DNA-binding regulatory protein that requires the brm/snr1 complex for its function in maintaining homeotic gene expression.

Studies of Human SWI/SNF Relatives Suggest Unanticipated Functions for the brm/snr1 Complex

Studies of mammalian homologues of brm and snr1 reveal involvement in cellular processes such as regulation of the cell cycle and viral integration. These functions may or may not be the result of transcriptional regulation by brm/snr1-related proteins. For example an unanticipated function of brg1 is its interaction with the retinoblastoma protein (Rb) in regulating progression of the cell cycle (Dunaief *et al.*, 1994). This suggests an additional role for the brm/snr1 complex in regulating cell division.

Studies of ini1, the human homologue of snr1, suggest a possible role in HIV proviral integration (Kalpana *et al.*, 1994). The *ini1* gene was isolated from a yeast two-hybrid screen by interaction with HIV integrase. Although the normal function of *ini1* is unknown, when tethered to DNA, ini1 is capable of activating transcription of a reporter gene (Kalpana *et al.*, 1994), suggesting that ini1, like SNF5, may function in transcription regulation. Biochemical evidence shows the interaction between ini1 and HIV integrase to be direct and that ini1 protein directly stimulates the integration reaction of integrase in vitro (Kalpana *et al.*, 1994). Like ini1, snr1 made in bacteria interacts with HIV integrase in vitro (our unpublished results), suggesting that snr1 and ini1 may be capable of interacting with a similar set of proteins. The SNF5 protein contains a 200-amino acid region that is highly similar to parts of snr1, ini1, and CeSNF5. This same region is sufficient for ini1 association with HIV integrase and may define a conserved domain necessary for protein-protein contacts. The interaction between integrase and ini1 probably does not represent a normal function of ini1; rather, the virus may have evolved to utilize ini1 to assist integration. The integration of the HIV viral genome into the host chromosome may be mediated by a direct interaction with ini1, either independently or within a human SWI/SNF-like complex (Kalpana *et al.*, 1994). Consistent with this idea, retroviruses have been shown to integrate preferentially into actively transcribed regions and their consequent open chromatin (Vijaya *et al.*, 1986; Rohdewold *et al.*, 1987; Shih *et al.*, 1988; Scherdin *et al.*, 1990). Alternatively, the integrase may persist at the site of integration and aid in attracting factors to allow transcription initiation.

The similarities between the yeast SWI/SNF complex and its *Drosophila* counterpart suggest that they may both be involved in gene regulation, albeit with different targets affected in different systems (Peterson and Tamkun, 1995). The unanticipated functions of the mammalian homologues of snr1 and brm suggest that either the fly and human proteins have evolved to interact with different proteins and/or that there is more than one SWI/SNF-like complex in higher eucaryotes. Although neither possibility can be ruled out, the existence of several *brm*-related genes in flies and humans is consistent with the idea that there are several SWI/SNF-like complexes (Carlson and Laurent, 1994; Elfring *et al.*, 1994). It seems likely that different complexes containing either snr1 or brm, or both, could act on different target genes, have different levels of activity, or have different types of protein-protein associations. Further biochemical characterization of the *Drosophila* brm/snr1 complex, its components, and possibly other related complexes, should provide a better understanding of the role of SWI/SNF relatives in patterning events in higher eukaryotes and lead to an elucidation of its role in gene expression and the maintenance of cell fates.

ACKNOWLEDGMENTS

We thank Dan Garza and Craig Peterson for expert technical advice and suggestions, Victoria Wang for help with fly stocks and crosses, and Lisa Elfring for advice and preparation of the brm antisera. We thank Craig Peterson and Fred Winston for critical reading of the manuscript. We also gratefully acknowledge the *Drosophila* Stock Center at Indiana University and T. Laverty and G. Rubin (*Drosophila* Genome Project, University of California, Berkeley) for providing P-element containing fly strains. A.K.D. and G.V.K. were supported by fellowships from the Helen Hay Whitney Foundation. This work was supported in part by NIH grant R01 GM-49883 to J.W.T. and in part by Public Health Service award U01 AI-24845 to S.P.G. Both S.P.G. and M.P.S. are investigators of the Howard Hughes Medical Institute.

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